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Activation of Kappa Opioid Receptors Inhibits Pruritus Evoked by Subcutaneous or Intrathecal Administration of Morphine in Monkeys

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Abstract

Pruritus (itch sensation) is the most common side effect associated with spinal administration of morphine given to humans for analgesia. A variety of agents have been proposed as antipruritics with poorly understood mechanisms and they are effective with variable success. *Kappa* opioid agonists possess several actions that are opposite to μ opioid agonists. We proposed to investigate the role of κ opioid receptors (KOR) in morphine-induced scratching and antinociception in monkeys. Scratching responses were counted by observers blinded to treatment. Antinociception was measured by a warm water (50^{o}C) tail-withdrawal assay. Pretreatment with low doses of U-50488H (0.032-0.18 mg/kg, s.c.), a selective KOR agonist, dose-dependently suppressed the s.c. morphine dose-effect curve for scratching and potentiated s.c. morphine-induced antinociception. In addition, s.c. U-50488H attenuated i.t. morphine (10 and 32 μg)-induced scratching while maintaining or enhancing i.t. morphine-induced antinociception. The combination of s.c. or i.t. morphine with low doses of U-50488H did not cause sedation. More importantly, pretreatment with 3.2 mg/kg of nor-binaltorphimine, a selective KOR antagonist, blocked the effects of s.c. U-50488H on both s.c. and i.t. morphine-induced scratching. These results indicate that activation of KOR attenuates morphine-induced scratching without interfering with antinociception in monkeys. This mechanism-based finding provides functional evidence in support of the clinical potential of KOR agonists as antipruritics in the presence of MOR agonist-induced pruritus.

Application of spinal opioids is one of the significant breakthroughs in pain management during the last two decades. For instance, intrathecal (i.t.) administration of morphine has been one of the most frequently used methods of analgesia following cesarean section and other surgical conditions. However, the most common side effect of spinal morphine is pruritus (i.e., itch sensation), which sometimes is severe and may lessen the value of spinal opioids for pain relief (Cousins and Mather, 1984; Ballantyne et al., 1988; Chaney, 1995; Kam and Tan, 1996). To date, there is no ideal antipruritic for patients. Several pharmacological agents have been proposed as antipruritics, but they are effective with variable success (Chaney, 1995; Kam and Tan, 1996). The mechanisms of proposed antipruritics are poorly understood, as there is a large deficiency in the basic research on spinal morphine-induced pruritus. This could be in part due to lack of reliable animal models. Although intracisternal administration of morphine evokes scratching responses in rodents, this behavior is not long-lasting and is only observed at small doses which do not produce profound antinociceptive effects (Tohda et al., 1997; Ko et al., 1999a). However, high doses of i.t. morphine produce allodynia-like behaviors, but no scratching, in rats. These behaviors are not reversed by opioid receptor antagonists (Yaksh and Harty, 1988).

Recently, we have established an experimental model of itch in monkeys (Ko and Naughton, 2000). We found that i.t. administration of morphine dose-dependently produced long-lasting scratching responses and thermal antinociception in these animals, and this parallels clinical observations (Baraka et al., 1981; Bailey et al., 1993; Palmer et al., 1999). In addition, results obtained from this model confirm the finding that activation of central μ opioid receptors (MOR) produces scratching behavior

(Thomas et al., 1992, 1993; Tohda et al., 1997; Kuraishi et al., 2000). This non-human primate itch model provides a valuable opportunity for itch research. It allows us to conduct further studies to elucidate both the mechanisms and potential treatments of MOR-induced pruritus.

Although opioid receptor antagonists are effective in attenuating i.t. morphine-induced pruritus, they are not ideal therapeutic agents for patients. Several clinical studies have shown that spinal opioid-mediated analgesia was reversed when high doses of opioid receptor antagonists were administered to treat pruritus (Cohen et al., 1992; Saiah et al., 1994; Wang et al., 1998). Our previous study also does not support opioid receptor antagonists for treatment of pruritus, because nalmefene produced an equal reduction of both scratching and antinociception in monkeys (Ko and Naughton, 2000). Thus, one important goal of our studies is to identify specific pharmacological agents that can attenuate morphine-induced pruritus without attenuating analgesia.

The κ opioid receptor (KOR) agonists appear to be a prominent potential target because several studies suggest that these agents may be therapeutically useful in this area. KOR agonists have antinociceptive effects, but possess several actions that are opposite to MOR agonists (e.g., Pan et al., 1998). One important finding is that behavioral profiles of monkeys with KOR agonist-dependence were qualitatively distinct from monkeys with morphine-dependence. Specifically, scratching is very prominent as a withdrawal sign in monkeys treated chronically with a selective KOR agonist, U-50488H (Gmerek et al., 1987). Many symptoms of withdrawal from opioids appear to be opposite to the acute effects of agonist administration. Excessive scratching activity indicates that acute administration of KOR agonists may have antipruritic function.

Studies in rodents seem to support this notion, as systemic administration of KOR agonists inhibits scratching behavior evoked by a variety of pruritogenic agents without interfering with locomotor activity (Cowan and Gmerek, 1986; Cowan and Kehner, 1997; Togashi et al., 2002). Given that there is a possible functional interaction between MOR and KOR, it is pivotal to investigate whether activation of KOR can suppress the itching sensation while maintaining or augmenting the antinociceptive effects of i.t. morphine in primate species.

The aim of this study was therefore to investigate the effects of systemic U-50488H on s.c. and i.t. administration of morphine for both scratching and antinociception. Moreover, pretreatment with a selective KOR antagonist, nor-binaltorphimine (nor-BNI), was conducted to verify whether KOR mediated the actions of U-50488H under these conditions.

Materials and Methods

Subjects

Twenty-eight adult intact male and female rhesus monkeys (*Macaca mulatta*) with body weights ranging between 6.1 and 11.3 kg were used. They were housed individually with free access to water and were fed approximately 25 to 30 biscuits (Purina Monkey Chow; Ralston Purina, St. Louis, MO) and fresh fruit daily. All monkeys were previously trained in the warm water tail-withdrawal procedure and no monkey had exposure to opioids for one month before the present study. The monkeys were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care. The studies were conducted in accordance with the University Committee on the Use and Care of Animals in the University of Michigan and the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington DC, revised 1996).

Procedures

I. Scratching responses

Scratching responses, inferred as an itch sensation (Ko and Naughton, 2000), were recorded on videotapes when monkeys were in their home cages. Each recording session was conducted for 15 min per test session. A scratch was defined as one short-duration (< 1sec) episode of scraping contact of the forepaw or hind paw on the skin surface of other body parts. Scratches usually occurred repetitively at the same location. Scratching responses were counted by experimenters who were blinded to experimental conditions.

II. Thermal antinociception

The warm water (50^{o}C) tail-withdrawal assay was used to evaluate thermal antinociceptive effects. Briefly, monkeys were seated in primate restraint chairs, and the lower part of their shaved tails (approximately 15 cm) were immersed in a thermal flask containing water maintained at either 40, 50, or 55^{o}C. Tail-withdrawal latencies were measured using a computerized timer by an experimenter who was blinded to experimental conditions. In each test session, monkeys were tested one to two times at three temperatures in a random order. If the monkeys did not remove their tails within 20 sec (cutoff), the flask was removed and a maximum time of 20 sec was recorded. Test sessions began with control determinations at each temperature. In addition, sedation was evaluated by an experimenter blind to experimental conditions based on a modified sedation rating scale before the measurement of tail-withdrawal latencies in each test session (Ko et al., 1999b).

III. Intrathecal injection

Monkeys were positioned in primate restraint chairs and anesthetized by i.v. propofol (2.5-4.0 mg/kg for bolus infusion and 0.3-0.4 mg/kg/min for continuous infusion; AstraZeneca, Wilmington, DE). The lower back of the trunk was shaved and prepared sterilely with Betadine. A spinal needle (22G x 1-1/2, Becton Dickinson, Franklin Lakes, NJ) was inserted into the subarachnoid space between L4/L5 lumbar vertebra. Needle position was confirmed by a free flow of clear cerebrospinal fluid. A 1-mL saline solution of morphine (10 or 32 μg) was infused slowly through the spinal needle within 30 sec. Monkeys recovered from anesthesia within 10 min following termination of propofol infusion.

Experimental Designs

The same group of monkeys, with the same number of each gender, were involved in both behavioral assays (i.e., scratching response and antinociception). However, both behavioral measurements were conducted in separate testing days. Each experimental condition associated with either behavioral assay was repeated one to two times, except with the antagonist study of nor-BNI.

I. Effects of U-50488H pretreatment

The first part of this study was to investigate effects of U-50488H pretreatment on the dose-effect curves of systemic morphine for both scratching and antinociception. Eight male and female monkeys were used in all experiments, which were conducted once per week. Morphine (0.1-3.2 mg/kg) was administered subcutaneously in the back using a cumulative dosing procedure with a 35-min inter-injection interval. Recording of scratching activity was performed from 20 to 35 min after each injection. On a separate experiment, tail-withdrawal latencies were determined starting at 20-25 min after each injection. U-50488H (0.032-0.18 mg/kg) or vehicle (sterile water, 0.1 mL/kg) was administered subcutaneously at 15 min before the injection of the first dose of morphine. Pretreatment doses of U-50488H including the vehicle condition were given in a random order.

II. Effects of U-50488H intervention

The second part of this study was to investigate whether U-50488H could reverse scratching subsequent to i.t. morphine administration. Two doses of morphine (10 and 32 μg) were chosen because these doses of i.t. morphine produced from moderate to maximum scratching responses (Ko and Naughton, 2000). Two groups of

monkeys were used (i.e., n=6 for one dose of i.t. morphine) for all experiments. Morphine was administered intrathecally with a 10-14-day inter-injection interval. Recording of scratching activity was conducted during 24-39th min and 54-09th min of each hour (i.e., 15 min per test session) for 3 hr after i.t. injection. During a separate experiment, tail-withdrawal latencies were measured at the same time points as the measurements of scratching. U-50488H (0.1-0.32 mg/kg) or vehicle (sterile water, 0.1 mL/kg) was administered subcutaneously at 45 min after i.t. injection of morphine. Similarly, intervention doses of U-50488H were given in a random order.

III. Antagonist effects of nor-BNI

The third part of the study investigated both the effects of nor-BNI alone and effects of nor-BNI on U-50488H modulation of behavioral effects produced by s.c. and i.t. morphine. First, nor-BNI (1-10 mg/kg) was administered subcutaneously to examine whether nor-BNI produced scratching responses. Doses of nor-BNI were chosen based on several antagonist studies indicating that they are systemically active doses in monkeys (Butelman et al., 1993, 1998). Three groups of monkeys were used (i.e., n=4 for one dose of nor-BNI) for all experiments. Recording of scratching was conducted during 24-39th min and 54-09th min of each hour for 3 hr after administration of nor-BNI. In addition, recording was carried out again for 3 hr at 24 hr after nor-BNI administration.

Second, pretreatment with nor-BNI was performed to examine whether nor-BNI would block the effects of U-50488H on s.c. morphine dose-effect curve for scratching. Based on data in the first part of this study, s.c. U-50488H 0.1 mg/kg significantly suppressed the dose-effect curve of s.c. morphine-induced scratching. This

experimental condition was further used for the nor-BNI study. There were two groups of monkeys (n=6 per group) for all experiments. One group received s.c. vehicle (sterile water, 0.1 ml/kg) and the other group received s.c. nor-BNI 3.2 mg/kg. Before monkeys received either vehicle or nor-BNI, each group of monkeys established their own control values of effects of U-50488H on s.c. morphine dose-effect curve for scratching. Effects of U-50488H on the dose-effect curve of morphine were re-determined at 1, 7, 14, 21, 28, and 35 days after treatment. These pretreatment time points were chosen based on studies indicating that peak KOR antagonist effects of nor-BNI were observed within the first two weeks after systemic administration in monkeys (Butelman et al., 1993, 1998).

Third, pretreatment with nor-BNI was performed to examine whether nor-BNI would alter the effects of U-50488H on i.t. morphine-induced scratching and antinociception. Based on data in the second part of this study, s.c. U-50488H 0.1 mg/kg significantly attenuated scratching and potentiated antinociception of i.t. morphine 10 μg. This experimental condition was further used for the nor-BNI study. There were two groups of monkeys (n=4 per group) for all experiments. One group received s.c. vehicle (sterile water, 0.1 ml/kg) and the other group received s.c. nor-BNI 3.2 mg/kg. Before monkeys received either treatment, each group of monkeys established their own control values of effects of U-50488H on i.t. morphine (10 μg)-induced scratching and antinociception. Effects of U-50488H on i.t. morphine-induced scratching and antinociception were re-determined at 1 day and 8 days after pretreatment, respectively.

Data Analysis

Mean values (mean ± S.E.M.) were calculated from individual values for all behavioral endpoints. Comparisons were made for the same monkeys across all test sessions in the same experiment. Data were analyzed by a two-way analysis of variance followed by the Newman-Keuls test for multiple (post hoc) comparisons ($p<0.05$ for significance). As noted, we did not find a significant difference in effects induced by morphine alone or morphine with U-50488H between male and female monkeys, so mean values for all monkeys in the same condition were used for data analysis. For the dose-effect curve of s.c. morphine-induced antinociception, individual tail-withdrawal latencies in 50^{o}C water were converted to percentage of maximum possible effect (%MPE). The formula of %MPE is defined as [(test latency – control latency)/(cutoff latency, 20 sec – control latency)] x 100. ED_{50} values were calculated by least-squares regression with the portion of the dose-effect curves spanning the 50% MPE. The 95% confidence limits (C.L.) were also determined ($p<0.05$). Mean ED_{50} values were considered to be significantly different when their 95% C.L. did not overlap.

Drugs

Morphine sulfate (Mallinckrodt, St. Louis, MO) was dissolved in saline. U-50488H (Sigma, St. Louis, MO) and nor-BNI (Div. Med. Chemistry, University of Michigan, Ann Arbor, MI) were dissolved in sterile water. For systemic administration, all compounds were administered subcutaneously in the back (i.e., around scapular region) at a volume of 0.1 ml/kg.

Results

I. Effects of U-50488H pretreatment

Figure 1 illustrates effects of U-50488H on the morphine dose-effect curves for scratching and antinociception. Systemic administration of morphine dose-dependently produced scratching responses [$F(3,21)=8.1$; $p<0.05$]. As noted, there was no sex difference under this condition [$F(1,3)=0.2$; $p>0.05$]. The mean value of s.c. morphine (1 mg/kg)-induced scratching was 375 ± 160 (S.E.M.) scratches in male monkeys (n=4); and the mean value for female monkeys (n=4) was 401 ± 104 scratches. The average number of scratches after a vehicle injection was 37 ± 13 for 15 min in this group of monkeys. Pretreatment with U-50488H attenuated the morphine dose-effect curve for scratching in a dose-dependent manner [$F(3,21)=19.0$; $p<0.05$]. Post hoc comparisons indicated that U-50488H from 0.032 to 0.18 mg/kg significantly attenuated the peak scratching effect of s.c. morphine 1 mg/kg (Figure 1, top panel).

Systemic morphine also dose-dependently produced antinociception against 50^{o}C water. Mean ED_{50} (95% C.L.) value of s.c. morphine-induced antinociception was 0.8 (0.5-1.4) mg/kg. As noted, mean ED_{50} value (0.6, 0.3-1.1 mg/kg) in male monkeys was not significantly different from the value (1.0, 0.3-3.2 mg/kg) in female monkeys. Pretreatment with U-50488H dose-dependently produced leftward shifts of the morphine dose-effect curve for antinociception (Figure 1, middle panel). In particular, s.c. U-50488H 0.18 mg/kg significantly produced a 6-fold leftward shift for morphine-induced antinociception (ED_{50}, 95% C.L.: 0.14, 0.1-0.3 mg/kg). In addition, pretreatment with U-50488H did not increase sedation under these conditions [$F(3,21)=1.5$; $p>0.05$] (Figure 1, bottom panel).

II. Effects of U-50488H intervention

Figure 2 illustrates effects of U-50488H on i.t. morphine-induced scratching and antinociception. Left panels show that s.c. U-50488H intervention dose-dependently attenuated i.t. morphine (10 μg)-induced scratching [$F(3,15)=22.4$; $p<0.05$]. Post hoc comparisons indicated that U-50488H (0.1-0.32 mg/kg) significantly attenuated scratching responses throughout the entire test sessions. In addition, s.c. U-50488H dose-dependently prolonged antinociception of i.t. morphine 10 μg [$F(3,15)=27.2$; $p<0.05$] and did not increase sedation rating under these conditions [$F(3,15)=2.6$; $p>0.05$].

Right panels illustrates effects of U-50488H on i.t. morphine (32 μg)-induced scratching and antinociception. I.t. morphine 32 μg produced more scratching responses and longer duration of antinociception than 10 μg of i.t. morphine. Similarly, s.c. U-50488H intervention dose-dependently attenuated i.t. morphine-induced scratching [$F(3,15)=19.4$; $p<0.05$] throughout the entire test sessions. In addition, s.c. U-50488H maintained i.t. morphine-induced antinociception [$F(3,15)=0.7$; $p>0.05$] and did not increase sedation rating under these conditions [$F(3,15)=2.7$; $p>0.05$]. Lack of U-50488H-induced enhancement of i.t. morphine (32 μg) may be due to a ceiling effect.

III. Antagonist effects of nor-BNI

As noted, nor-BNI (1-10 mg/kg) did not produce scratching responses during the first 3 hr following s.c. administration [$F(3,9)=3.4$; $p>0.05$]. Nor-BNI also did not increase scratching responses at 24 hr after s.c. administration [$F(3,9)=0.6$; $p>0.05$] (data not shown). Figure 3 illustrates the antagonist effect of nor-BNI on the actions of U-50488H against s.c. morphine. Pretreatment with vehicle did not change the effect of U-50488H

on s.c. morphine dose-effect curve for scratching throughout the entire study period [$F(6,30)=1.7$; $p>0.05$] (Figure 3, panels A and B). In contrast, pretreatment with nor-BNI 3.2 mg/kg significantly blocked attenuation of U-50488H on s.c. morphine dose-effect curve for scratching in a time-dependent manner [$F(6,30)=4.3$; $p<0.05$]. Post hoc comparisons indicated that nor-BNI 3.2 mg/kg significantly blocked the actions of U-50488H 0.1 mg/kg from 1 day to 21 days following treatment (Figure 3, panels C and D).

Figure 4 illustrates the antagonist effect of nor-BNI on the actions of U-50488H against i.t. morphine. Pretreatment with vehicle did not change the effect of U-50488H on i.t. morphine (10 μg)-induced scratching responses [$F(1,3)=0.2$; $p>0.05$]. Vehicle also did not change the effect of U-50488H on i.t. morphine (10 μg)-induced antinociception [$F(1,3)=0.3$; $p>0.05$] (Figure 4, panels A and B). In contrast, pretreatment with nor-BNI 3.2 mg/kg significantly blocked attenuation of U-50488H on i.t. morphine-induced scratching [$F(1,3)=129$; $p<0.05$]. Post hoc comparisons indicated that U-50488H 0.1 mg/kg could not attenuate scratching for the entire sessions (Figure 4, panel C). In addition, nor-BNI pretreatment also blocked U-50488H-induced potentiation of i.t. morphine-induced antinociception [$F(1,3)=13.2$; $p<0.05$] (Figure 4, panel D).

Discussion

The present study demonstrated that systemic administration of low doses of U-50488H could either prevent or attenuate morphine-induced scratching behavior. In particular, systemic U-50488H attenuated i.t. morphine-induced scratching while maintaining or enhancing morphine-induced antinociception. The actions of U-50488H were mediated by activation of KOR. This is the first study to validate the effectiveness of KOR activation in attenuating morphine-mediated scratching responses in primate species.

The dose-effect curve of s.c. morphine for scratching behavior can be established in monkeys. It is worth noting a large potency difference (i.e., ~1000 fold) between s.c. and i.t. morphine-induced scratching. The peak pruritic effect of s.c. morphine is observed approximately at 1 mg/kg, which equals to 10 mg total dose for monkeys with mean body weight of 10 kg in our laboratory. In contrast, the dose of i.t. morphine for producing a similar degree of scratching responses is approximately 10 μg (see Figure 2 in this study; Ko and Naughton, 2000). The hydrophilic property and associated difficulty crossing the blood-brain barrier of morphine may be part of the reason for this phenomenon (Herz and Teschemacher, 1971). It will be valuable to further compare the relative potency in evoking scratching between s.c. and i.t. administration of MOR agonists with different lipophilicities. Nevertheless, a large relative potency between s.c. and i.t. morphine-induced scratching may indicate that activation of central MOR mediates scratching behavior (Thomas et al., 1992, 1993; Tohda et al., 1997; Kuraishi et al., 2000).

Pretreatment with U-50488H suppressed the s.c. morphine dose-effect curve for scratching. The interaction between U-50488H and morphine in scratching is not mediated by MOR antagonism, but may be a functional antagonism. KOR agonists possess several actions opposite to MOR agonists (e.g., Pan et al., 1998). The potential antipruritic action of KOR may counteract the pruritic action of MOR. The opposite functional interaction between MOR and KOR has been reported with other *in vivo* evidence. For example, KOR agonists produce diuretic effects and MOR agonists produce antidiuretic effects. It has been shown that full KOR agonists with MOR agonist activities, but not pure KOR agonists, have inverted U-shaped dose-effect curves for diuresis (Leander, 1984).

Pretreatment with U-50488H potentiated morphine-induced antinociception as indicated by leftward shifts of the morphine dose-effect curve. Interestingly, another study in monkeys found that pretreatment with morphine produced a leftward shift of U-50488H dose-effect curve for antinociception (Butelman et al., 1995). Several studies have shown that systemic administration of either MOR or KOR agonists produces antinociceptive effects in monkeys (Butelman et al., 1993, 1998; France et al., 1994; Ko et al., 1998, 1999b). Both MOR and KOR may produce antinociception through a common mechanism by disinhibition of pain inhibitory neurons (Ackley et al., 2001). It seems important to further investigate whether U-50488H enhances antinociception produced by other MOR agonists in monkeys.

Systemic administration of U-50488H attenuated i.t. morphine-induced scratching responses. More importantly, s.c. U-50488H maintained or prolonged i.t. morphine-induced antinociception without enhancing sedation. As noted, doses of U-50488H (i.e.,

0.032-0.32 mg/kg) used in the present study are low doses, which do not produce significant antinociception and sedation in monkeys when administered alone. Higher doses of U-50488H (i.e., ≤0.56-3.2 mg/kg) produce moderate to full antinociception in the presence of sedation and muscle relaxation (Butelman et al., 1993, 1998; France et al., 1994; Ko et al., 1998, 1999b). In addition, given combination of U-50488H with either s.c. morphine or i.t. morphine, monkeys did not have observable muscle relaxation. These results agree with the finding that KOR agonists inhibit scratching at doses that do not suppress locomotor activity and general behavior in rodents (Cowan and Kehner, 1997; Togashi et al., 2002). Nevertheless, 0.1 mg/kg of U-50488H is detectable for monkeys trained to discriminate KOR agonists (France et al., 1994). It is possible that KOR-mediated dysphoria may limit its therapeutic potential. Nevertheless, MOR-mediated euphoria may counteract KOR-mediated dysphoria. An opioid agonist with mixed actions of high efficacy on MOR and low efficacy on KOR may be an ideal candidate as an analgesic with low abuse liability. Unfortunately, to date, there is no such a compound available for experimental research in animals. Mixed MOR/KOR agonists such as butorphanol do not follow this profile, as butorphanol has low to medium efficacy on both MOR and KOR (Butelman et al., 1995).

Nor-BNI did not evoke scratching responses in monkeys immediately or 24 hr after systemic administration. This finding does not agree with the results of a rodent study indicating that nor-BNI-induced scratching behavior is partially mediated by the release of histamine (Kamei and Nagase, 2001). There are several factors that may contribute to this functional discrepancy across species. For example, there are species

differences in KOR regulation. A study has shown differences in KOR agonist-induced desensitization and phosphorylation between human KOR and rat KOR (Li et al., 2002). In addition, a newly developed KOR antagonist, GNTI, also produced profound scratching responses in mice. However, pretreatment with KOR agonists only partially attenuated GNTI-induced scratching, indicating a potential role of non-KOR (Cowan et al., 2002).

The KOR antagonist effect of s.c. nor-BNI can be clearly detected at 24 hr following administration and it lasts for 2-3 weeks in monkeys (Butelman et al., 1993, 1998). The time course of nor-BNI-induced KOR antagonism in this study is similar to previous findings. Pretreatment with nor-BNI 3.2 mg/kg produced a 21-day antagonism for the actions of U-50488H on the s.c. morphine dose-effect curve for scratching (Figure 3). In addition, pretreatment with the same dose of nor-BNI completely blocked the actions of U-50488H on i.t. morphine-induced scratching and antinociception (Figure 4). These results confirm that effects of U-50488H are mediated by KOR and may be a mechanism-based finding for KOR agonists as potential antipruritics. Future studies are needed to investigate the effects of i.t. administration of combination of morphine and U-50488H to determine the sites of action of KOR agonists as antipruritics. Moreover, it is important to compare the effectiveness of KOR agonists with less ability to enter CNS with prototypic KOR agonist U-50488H as antipruritics (Cowan and Gmerek, 1986; Cowan and Kehner, 1997).

Although morphine can release histamine from mast cells, this may not be its main mechanism of pruritus. Several studies have shown that antihistamines are not effective in relieving morphine-induced pruritus (Thomas et al., 1993; Dunteman et al.,

1996; Kam and Tan, 1996). Moreover, not all MOR agonists produce histamine release in humans. Intravenous administration of MOR agonists, such as fentanyl, produced itch sensation, but they did not induce histamine release (Hermens et al., 1985; Flacke et al., 1987; Stellato et al., 1992). Although a class of spinothalamic tract neurons selectively sensitive to histamine has been identified (Andrew and Craig, 2001), it seems more pivotal to identify the sensory neurons in which MOR and KOR are co-localized in primate species (Gutstein et al., 1998).

In summary, this study demonstrates the effectiveness of KOR activation in attenuating morphine-induced scratching responses in non-human primates. It may be useful to use KOR agonists to treat pruritus associated with spinal administration of MOR agonists in humans.

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References

Ackley MA, Hurley RW, Virnich DE and Hammond DL (2001) A cellular mechanism for the antinociceptive effect of a kappa opioid receptor agonist. *Pain* **91**:377-388.

Andrew D and Craig AD (2001) Spinothalamic lamina I neurons selectively sensitive to histamine: a central neural pathway for itch. *Nat Neurosci* **4**:72-77.

Bailey PL, Rhondeau S, Schafer PG, Lu JK, Timmins BS, Foster W, Pace NL and Stanley TH (1993) Dose-response pharmacology of intrathecal morphine in human volunteers. *Anesthesiology* **79**:49-59.

Ballantyne JC, Loach AB and Carr DB (1988) Itching after epidural and spinal opiates. *Pain* **33**:149-160.

Baraka A, Noueihid R and Hajj S (1981) Intrathecal injection of morphine for obstetric analgesia. *Anesthesiology* **54**:136-140.

Butelman ER, Negus SS, Ai Y, de Costa BR and Woods JH (1993) Kappa opioid antagonist effects of systemically administered nor-binaltorphimine in a thermal antinociception assay in rhesus monkeys. *J Pharmacol Exp Ther* **267**:1269-1276.

Butelman ER, Winger G, Zernig G and Woods JH (1995) Butorphanol: Characterization of agonist and antagonist effects in rhesus monkeys. *J Pharmacol Exp Ther* **272**:845-853.

Butelman ER, Ko MC, Sobczyk-kojiko K, Mosberg HI, Van Bemmel B, Zernig G and Woods JH (1998) Kappa-opioid receptor binding populations in rhesus monkey brain: Relationship to an assay of thermal antinociception. *J Pharmacol Exp Ther* **285**:595-601.

Chaney MA (1995) Side effects of intrathecal and epidural opioids. *Can J Anaesth*

42:891-903.

Cohen SE, Ratner EF, Kreitzman TR, Archer JH and Mignano LR (1992) Nalbuphine is better than naloxone for treatment of side effects after epidural morphine. *Anesth Analg* **75**:747-752.

Cousins MJ and Mather LE (1984) Intrathecal and epidural administration of opioids. *Anesthesiology* **61**:276-310.

Cowan A and Gmerek DE (1986) *In-vivo* studies on kappa opioid receptors. *Tr Pharmacol Sci* **7**:69-72.

Cowan A and Kehner GB (1997) Antagonism by opioids of compound 48/80-induced scratching in mice. *Br J Pharmacol* **122(suppl)**:p.169.

Cowan A, Inan S and Kehner GB (2002) GNTI, a kappa receptor antagonist, causes compulsive scratching in mice. *The Pharmacologist* **44(suppl):**A51.

Dunteman E, Karanikolas M and Filos KS (1996) Transnasal butorphanol for the treatment of opioid-induced pruritus unresponsive to antihistamines. *J Pain Symptom Manage* **12**:255-260.

Flacke JW, Flacke WE, Bloor BC, Van Etten AP and Kripke BJ (1987) Histamine release by four narcotics: a double-blind study in humans. *Anesth Analg* **66**:723-730.

France CP, Medzihradsky F and Woods JH (1994) Comparison of kappa opioids in rhesus monkeys: behavioral effects and receptor binding affinities. *J Pharmacol Exp Ther* **268**:47-58.

Gmerek DE, Dykstra LA and Woods JH (1987) Kappa opioids in rhesus monkeys. III. Dependence associated with chronic administration. *J Pharmacol Exp Ther*

242:428-436.

Gutstein HB, Mansour A, Watson SJ, Akil H and Fields HL (1998) Mu and kappa opioid receptors in periaqueductal gray and rostral ventromedial medulla. *NeuroReport* **9**:1777-1781.

Hermens JM, Ebertz JM, Hanifin JM and Hirshman CA (1985) Comparison of histamine release in human skin mast cells induced by morphine, fentanyl, and oxymorphone. *Anesthesiology* **62**:124-129.

Herz A and Teschemacher HJ (1971) Activities and sites of antinociceptive action of morphine-like analgesics and kinetics of distribution following intravenous, intracerebral and intraventricular application, in *Advances in drug research* (Harper NJ and Simmonds AB eds) pp 79-119, Academic Press, New York.

Kam PCA and Tan KH (1996) Pruritus - itching for a cause and relief? *Anaesthesia* **51**:1133-1138.

Kamei J and Nagase H (2001) Norbinaltorphimine, a selective kappa-opioid receptor antagonist, induces an itch-associated response in mice. *Eur J Pharmacol* **418**:141-145.

Ko MC, Butelman ER, Traynor JR and Woods JH (1998) Differentiation of kappa opioid agonist-induced antinociception by naltrexone apparent pA2 analysis in rhesus monkeys. *J Pharmacol Exp Ther* **285**:518-526.

Ko MC, Johnson MD, Choo KS, Song MS, Chang D, Naughton NN and Woods JH (1999a) An experimental itch model in rats: Characterization of intracisternal morphine-induced scratching and antinociception. *FASEB Journal* **13**:622.611.

Ko MCH, Johnson MD, Butelman ER, Willmont KJ, Mosberg HI and Woods JH (1999b)

Intracisternal nor-binaltorphimine distinguishes central and peripheral kappa-opioid antinociception in rhesus monkeys. *J Pharmacol Exp Ther* **291**:1113-1120.

Ko MCH and Naughton NN (2000) An experimental itch model in monkeys: Characterization of intrathecal morphine-induced scratching and antinociception. *Anesthesiology* **92**:795-805.

Kuraishi Y, Yamaguchi T and Miyamoto T (2000) Itch-scratch responses induced by opioids through central mu opioid receptors in mice. *J Biomed Sci* **7**:248-252.

Leander JD (1984) Effects of full and partial kappa agonists and mu agonists on urine output of normally hydrated rats. *Neuropeptides* **5**:283-286.

Li J, Li JG, Chen C, Zhang F and Liu-Chen LY (2002) Molecular basis of differences in (-)(trans)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidiny)-cyclohexyl]benzeneac etamide-induced desensitization and phosphorylation between human and rat kappa-opioid receptors expressed in Chinese hamster ovary cells. *Mol Pharmacol* **61**:73-84.

Palmer CM, Emerson S, Volgoropolous D and Alves D (1999) Dose-response relationship of intrathecal morphine for postcesarean analgesia. *Anesthesiology* **90**:437-444.

Pan ZZ (1998) mu-Opposing actions of the kappa-opioid receptor. *Trends Pharmacol Sci* **19**:94-98.

Saiah M, Borgeat A, Wilder-Smith OH, Rifat K and Suter PM (1994) Epidural-morphine-induced pruritus: propofol versus naloxone. *Anesth Analg* **78**:1110-1113.

Stellato C, Cirillo R, de Paulis A, Casolaro V, Patella V, Mastronardi P, Mazzarella B and Marone G (1992) Human basophil / Mast cell releasability. *Anesthesiology*

77:932-940.

Thomas DA, Williams GM, Iwata K, Kenshalo DR, Jr. and Dubner R (1992) Effects of central administration of opioids on facial scratching in monkeys. *Brain Res* **585**:315-317.

Thomas DA, Williams GM, Iwata K, Kenshalo DR, Jr. and Dubner R (1993) The medullary dorsal horn. A site of action of morphine in producing facial scratching in monkeys. *Anesthesiology* **79**:548-554.

Togashi Y, Umeuchi H, Okano K, Ando N, Yoshizawa Y, Honda T, Kawamura K, Endoh T, Utsumi J, Kamei J, Tanaka T and Nagase H (2002) Antipruritic activity of the κ-opioid receptor agonist, TRK-820. *Eur J Pharmacol* **435**:259-264.

Tohda C, Yamaguchi T and Kuraishi Y (1997) Intracisternal injection of opioids induces itch-associated response through mu-opioid receptors in mice. *Jpn J Pharmacol* **74**:77-82.

Wang JJ, Ho ST and Tzeng JI (1998) Comparison of intravenous nalbuphine infusion versus naloxone in the prevention of epidural morphine-related side effects. *Reg Anesth Pain Med* **23**:479-484.

Yaksh TL and Harty GJ (1988) Pharmacology of the allodynia in rats evoked by high dose intrathecal morphine. *J Pharmacol Exp Ther* **244**:501-507.

Footnotes

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Figure Legends

Figure 1. Effects of U-50488H pretreatment on systemic morphine dose-effect curves for scratching responses and antinociception. Top panel shows the effect of U-50488H on s.c. morphine-induced scratching. Middle and bottom panels show the effect of U-50488H on s.c. morphine-induced antinociception and sedation, respectively. U-50488H (mg/kg) was given subcutaneously at 15 min before the first dose of morphine. Each value represents mean ± S.E.M. (n=8). Symbols represent different experimental conditions for the same monkeys. The asterisk represents a significant difference from the vehicle condition (∗ $p<0.05$).

Figure 2. Effects of U-50488H intervention on i.t. morphine-induced scratching responses and antinociception. Left panels show the effect of U-50488H on i.t. morphine (10 μg)-induced scratching, antinociception, and sedation. Similarly, right panels show the effect of U-50488H on i.t. morphine (32 μg)-induced scratching, antinociception, and sedation. Antinociception was measured by a warm water (50^{o}C) tail-withdrawal procedure. U-50488H was given subcutaneously at 45 min after i.t. injection of morphine. Each value represents mean ± S.E.M. (n=6). Symbols represent different experimental conditions for the same monkeys. The asterisk represents a significant difference from the vehicle condition between time points 1 hr and 2.5 hr (∗ $p<0.05$). The symbol (#) associated with data points represents a significant difference ($p<0.05$) from the vehicle condition between 2hr and 2.5 hr.

Figure 3. The antagonist effect of nor-BNI on the actions of U-50488H against s.c. morphine. Panels A and B show the effect of s.c. vehicle pretreatment (PT) on the actions of U-50488H. Panels C and D show the effect of s.c. nor-BNI PT on the actions of U-50488H. U-50488H 0.1 mg/kg was given subcutaneously at 15 min before the first dose of morphine. The dose-effect curve (DEC) of morphine-induced scratching was determined at 1, 7, 14, 21, 28, 35 days after PT. Each value represents mean ± S.E.M. (n=6). CTRL represents the effects of U-50488H on the DEC of morphine-induced scratching before each group of monkeys (n=6) received either vehicle or nor-BNI. The asterisk represents a significant difference from the CTRL condition (∗ $p<0.05$). See Experimental Designs and Figure 1 for other details.

Figure 4. The antagonist effect of nor-BNI on the actions of U-50488H against i.t. morphine. Panels A and B show the effect of s.c. vehicle pretreatment (PT) on the actions of U-50488H. Panels C and D show the effect of s.c. nor-BNI PT on the actions of U-50488H. U-50488H 0.1 mg/kg was given subcutaneously at 45 min after i.t. injection of morphine. I.t. morphine (10 μg)-induced scratching and antinociception were determined at 1 and 8 days after PT, respectively. Each value represents mean ± S.E.M. (n=4). CTRL represents the effects of U-50488H on i.t. morphine-induced scratching and antinociception before each group of monkeys (n=4) received either vehicle or nor-BNI. The asterisk represents a significant difference from the CTRL condition (∗ $p<0.05$). See Experimental Designs and Figure 2 for other details.

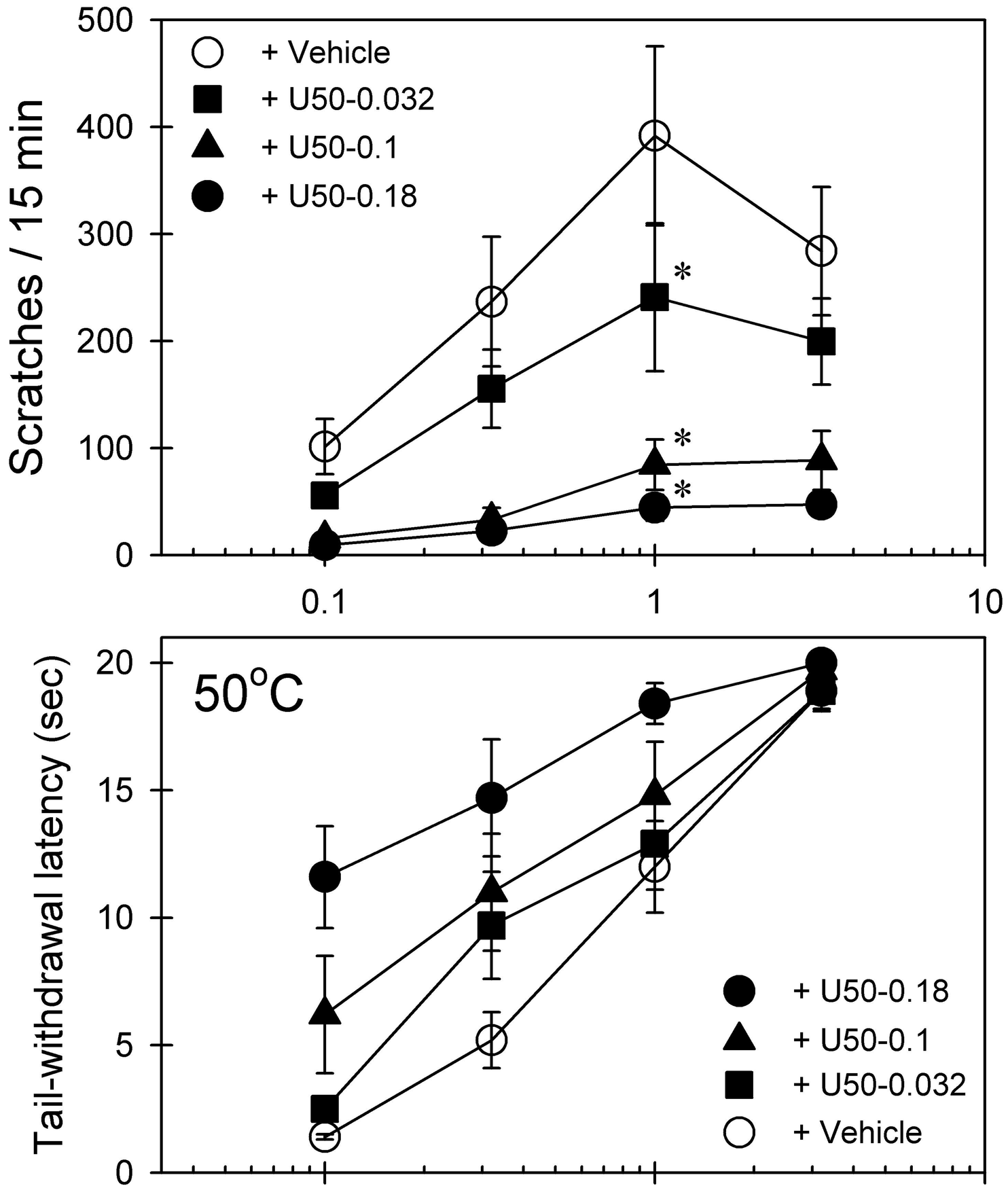
Scratches / 15 min
500
400
300
200
100
0
+ Vehicle
+ U50-0.032
+ U50-0.1
+ U50-0.18
*
*
*
0.1
1
10
Tail-withdrawal latency (sec)
20
15
10
5
0
50°C
+ U50-0.18
+ U50-0.1
+ U50-0.032
+ Vehicle
0.1
1
10
Sedation rating
3
2
1
0
+ Vehicle
+ U50-0.032
+ U50-0.1
+ U50-0.18
0.1
1
10
Dose of morphine (mg/kg, s.c.)

FIG 1-Ko et al.

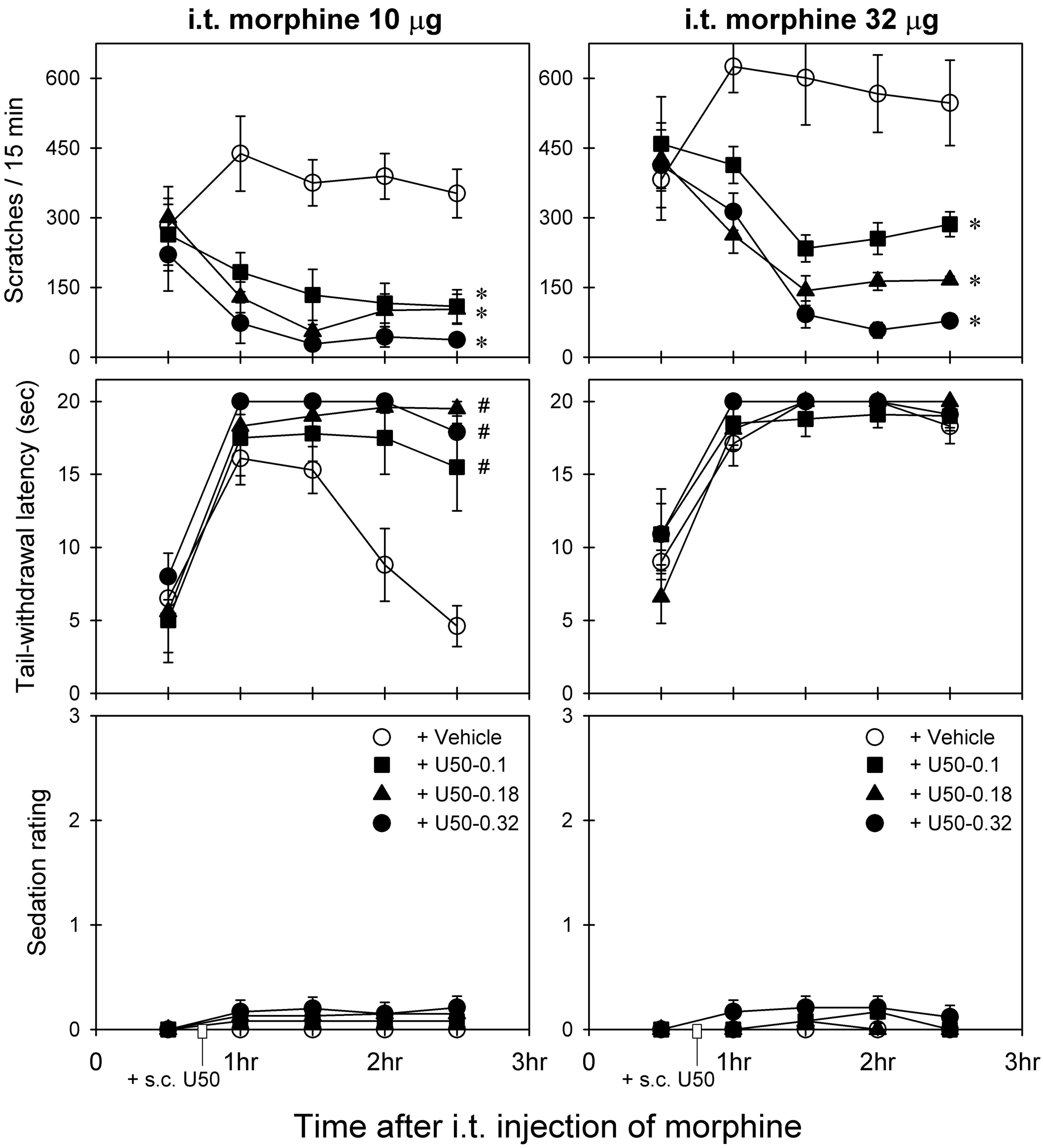
i.t. morphine 10 μg
i.t. morphine 32 μg
Scratches / 15 min
600
450
300
150
0
*
*
*
Tail-withdrawal latency (sec)
20
15
10
5
0
#
#
#
Sedation rating
3
2
1
0
+ Vehicle
+ U50-0.1
+ U50-0.18
+ U50-0.32
0
1hr
2hr
3hr
+ s.c. U50
Time after i.t. injection of morphine

FIG 2-Ko et al.

+ vehicle 0.1 ml/kg

+ nor-BNI 3.2 mg/kg

A

B

C

D

CTRL

1-day PT

7-day PT

14-day PT

21-day PT

35-day PT

Scratches / 15 min

0

100

200

300

400

500

0.1

1

10

*

+ s.c. U50 0.1 mg/kg
15 min before morphine DEC

Dose of morphine (mg/kg, s.c.)

FIG 3-Ko et al.

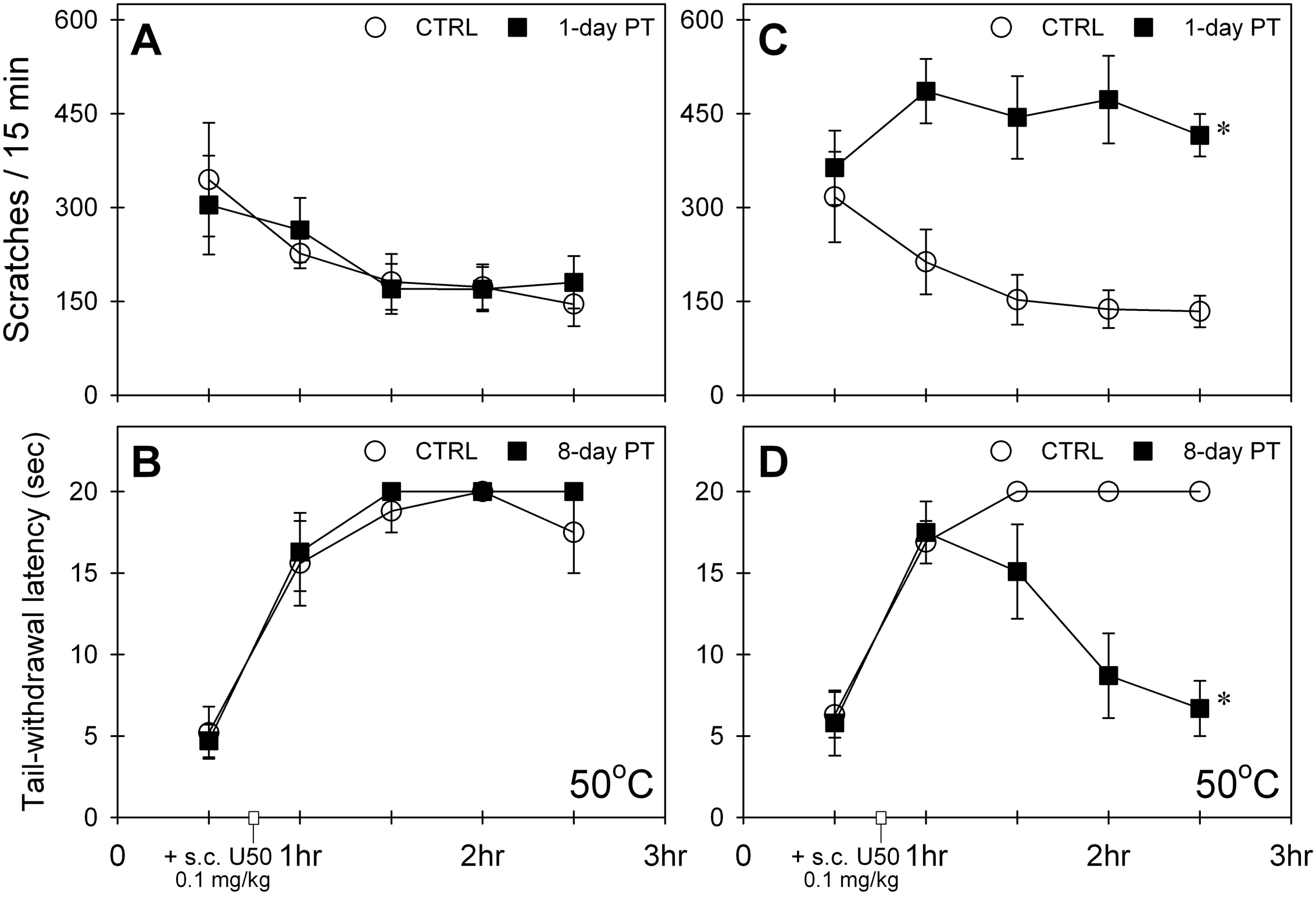
+ vehicle 0.1 ml/kg
+ nor-BNI 3.2 mg/kg
A
B
C
D
CTRL
1-day PT
8-day PT
Scratches / 15 min
Tail-withdrawal latency (sec)
600
450
300
150
0
20
15
10
5
50°C
0
+ s.c. U50
0.1 mg/kg
1hr
2hr
3hr
*
Time after i.t. injection of morphine 10 μg

FIG 4-Ko et al.